Assessing persistence of chemicals in marine water using biodegradation tests: impact of the testing conditions on results and regulatory decisions

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Introduction

In order to assess the persistency of chemicals in marine water, biodegradation tests performed according to the standard guidelines are commonly required by the authorities. According to the regulatory requirements some deviations to the standard guidelines are allowed and, in 2016 a new temperature of 12°C has been recommended under REACH for simulation test. Simultaneously, recent results from CEFIC-LRi funded projects (ECO11 and ECO18) have shown that the test conditions could have a major impact on the estimated biodegradation rates and, as a consequence, on the regulatory decisions for the tested substances.

In order to assess at which extent the test design is affecting the biodegradation in marine water of one of our organic substance, multiple tests using different conditions have been carried out in the same laboratory. The objective was to critically compared the results according to their environmental relevance and their regulatory consequences under various chemical regulations.

Material & Methods

Five marine biodegradation tests have been performed according to the **Close bottle test method** of the **OECD 306 guideline** on our test substance at **low concentration** (1 mg/L) with the following adaptations:

- **extended duration** up to 60 days (standard conditions)
- **semi-continuous** regime (1/3 water replacement at day 14)
- **higher test substance concentration** (3 mg/L)
- **12°C** testing temperature
- **higher testing volume** (4 folds)

In order to assess the seasonal variation of biodegradation at a same location, the results of a test performed **one year before** under the same standard conditions have been added to the dataset (represented as 1 year before series).

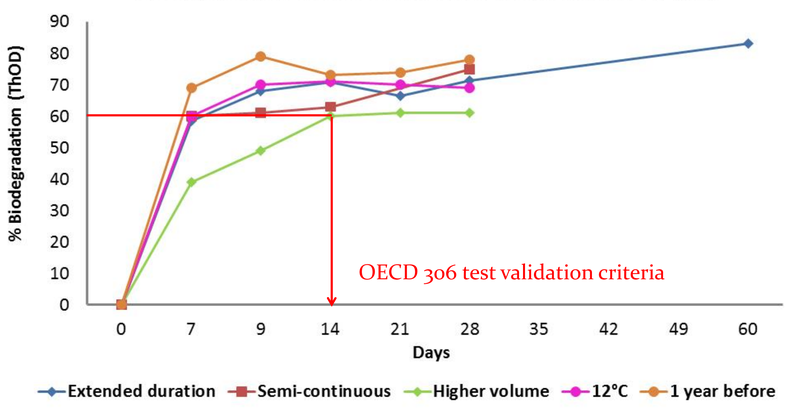
In all the tests, a **rapidly degradable reference substance** (sodium benzoate at 2mg/L) has been tested in parallel under the same conditions (except higher concentration).

The total **microbial viable count** has been measured as Colony Forming Unit (CFU/mL) on the collected water.

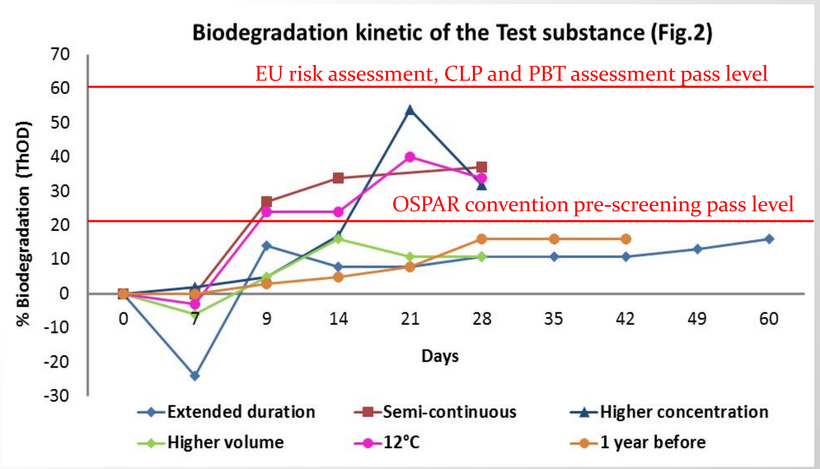
The biodegradation rate results have been statistically analyzed by **multiple-comparison procedures**.

Results and Discussion

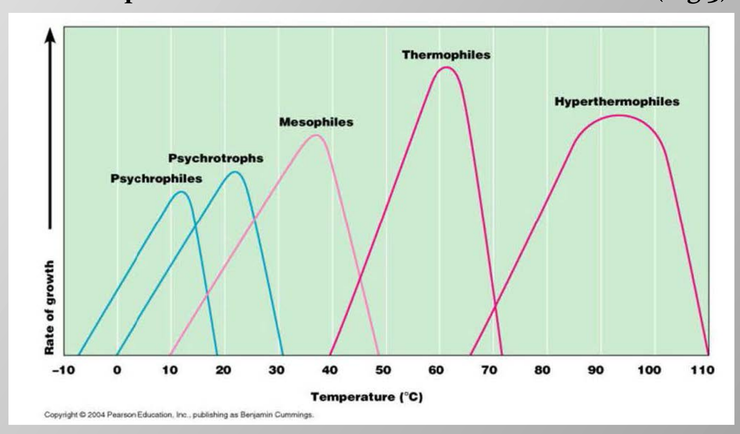
Biodegradation kinetic of the Reference substance (Fig.1)



Biodegradation kinetic of the Test substance (Fig.2)



Growth optimum T° for different classes of microbes (Fig.3)



Effect of the testing conditions on biodegradation

- **Test duration:** If the extension of the exposure period up to 60 days has allowed an acceleration of the reference substance biodegradation (Fig.1), suggesting an **adaptation of the micro-organisms** after 28 days, it is not the case for the less rapidly biodegradable test substance (Fig.2).
- **Semi-continuous regime:** a semi-continuous regime had mainly improved the biodegradation of the test substance (Fig.2)and didn't significantly impacted the biodegradation of the reference substance (Fig.1). The microbial count of the replacement water being four times below the one of the original water, we assumed that the biodegradation enhancement was due to the introduction of **fresh competent degraders** with the replacement water. This phenomena occurring naturally in the environment, we wonder why this test adaptation of the OECD 306 guideline is not accepted by the authorities, especially when semi-continuous regime is permitted in biodegradation simulation tests for marine waters (OECD 309)?
- **Test substance concentration:** testing a 3 orders of magnitude higher concentration of the tested substance has increased its biodegradation profile (Fig.2) almost reaching the 60% biodegradation pass level. This is probably because **microbial growth** could occurred with this higher level of organic carbon, when it is generally assumed to be limited under simulated degradation conditions. Therefore, we believe that an applicant should consider whether to launch a simulation test, which is offering more testing adaptations, rather than a screening biodegradation test at low concentration, especially if the synthesis of a radio-labelled test substance is required.
- **Temperature:** marine water was collected at 17°C (8°C 1 year before). Performing the test at 12°C (instead of 19°C in all other tests) didn't modified the biodegradation profile of the reference substance (Fig.1) probably due to the presence of multiple competent microbial communities for such readily degradable substance. Instead, the biodegradation kinetic of the tested substance has been improved at 12°C (Fig.2) suggesting a **more stringent variety of competent degraders** most probably belonging to the Psychrophiles class of microbes surviving and proliferating at low temperatures with a growth optimum around 12°C (Fig.3).
- **Test volume:** surprisingly, increasing the test volume, and consequently the probability of introducing competent microorganisms into the test vessel, didn't have improved the biodegradation of the test substance (Fig.2) and has even decreased the biodegradation of the reference substance jeopardizing the test validation (Fig.1)! It seems to be due to a modification of the **respiration baseline of the microorganisms** in the control series.
- **Seasonal effect:** a standard test performed one year before during the same season (August) didn't shown an impact on the biodegradation profiles of the test and reference substances (Fig.1&2) suggesting a **conservation of the competent degraders communities at the same location** even if the microbial count and the water temperature of the collected water were two times lowers (760 & 1450 CFU/mL - 8 & 17°C, respectively).

Conclusion

None of the tested conditions has allowed to reach the 60% ThOD removal threshold for the test substance indicative of potential for ultimate biodegradation in the marine environment for **Risk assessment**, **Classification and Labelling** and **PBT assessment** purposes. However, testing under **semi-continuous regime, at higher concentration and at 12°C**, has allowed to pass the **20% biodegradation pre-screening threshold** for the authorisation of offshore products under the **OSPAR convention**. Interestingly, these results are not always supported by statistical analysis: test substance biodegradation improvement under these three conditions is significant with the Ryan-Einot- Gabriel-Welsch multiple range test but not with the Scheffe's test. This means that we have not enough data and/or not enough precise measures to control the "overall significance level" of inferences, the latest possibly being caused by the **decreased reliability of the biodegradability assessment at low substance concentration.**

These three conditions will be used to conduct a future surface water simulation test with a radio-labelled test substance (OECD 309).

A comparison with the reference substance results shows that the **impact of the testing conditions will be globally less important on ready biodegradable substances**, probably because they could be mineralised by more microorganisms classes procuring a higher level of adaptation to different conditions.

As a remark, we noticed that the microbial count recommended in the guideline is not a good indicator of competent degraders in the collected marine water.

Finally, we recommend to consider the impact of the testing conditions before launching a new marine biodegradation test in order to define an **optimized study plan in the limit of the regulatory accepted adaptations**.

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